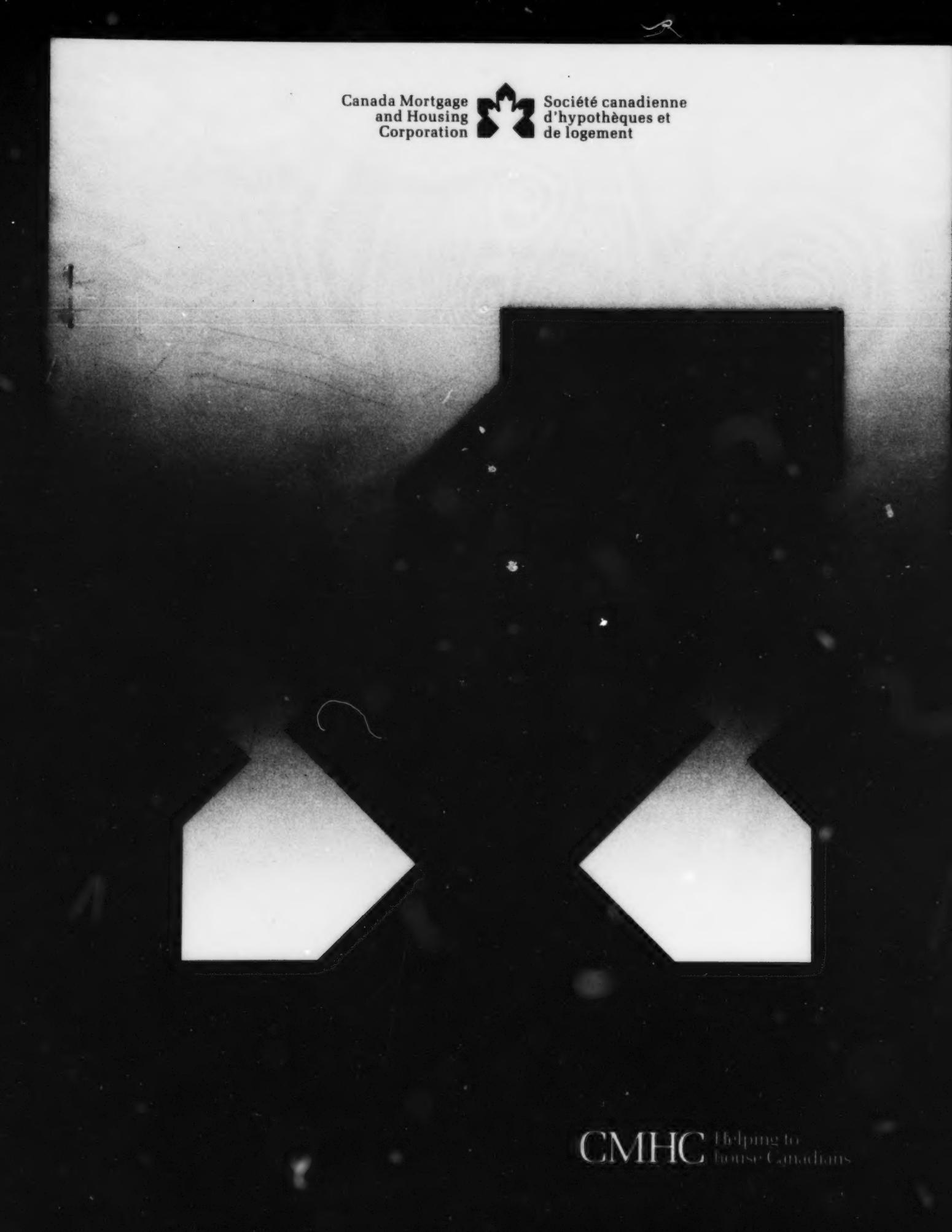
Canada Mortgage and Housing Corporation
Société canadienne d'hypothèques et de logement
CMHC
Helping to house Canadians

DETERMINATION OF FUNGAL PROPAGALES IN INDOOR AIR

PROJECT REPORT

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Canada Mortgage and Housing Corporation, the Federal Goverment's housing agency, is responsible for administering the National Housing Act.

This legislation is designed to aid in the improvement of housing and living in Canada. As a result, the Corporation has interests in all aspects of housing and urban growth and development.

Under Part V of this Act, the Government of Canada provides funds to CMHC to conduct research into the social, economic and technical aspects of housing and related fields, and to undertake the publishing and distribution of the results of this research. CMHC therefore has a statutory responsability to make widely available, information which may be useful in the improvement of housing and living conditions.

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1. The Problem

Fungi as spores and mycelial fragments are found in air and, to the extent that these are phylloplane (originating on the surface of leaves), this is usually normal. Fungal spores come in a great variety of sizes, shapes, and adhesivities; thus, they do not sediment in air equally. Inhalation of fungal spores of different species of fungi produce different health effects: some are pathogens (*e.g., Aspergillus fumigatus*); some contain mycotoxins (*e.g., Stachybotris atra*); most, if not all, produce allergens, and inhalation of large number of spores of any fungus will produce hypersensitivity pneumonitis (1).

Identification of the fungi present to species is critical to an unequivocal determination of a hazardous situation with respect to fungi in indoor air. Unfortunately, this is usually impractical because of the limited availability of the relevant expertise and the considerable expense involved. The purpose of this method is to describe an evaluation strategy to assess fungal contamination of indoor air by measurement and inspection. The approach outlined will provide a satisfactory indication of the extent of fungal contamination of indoor air under most circumstances. Continued unexplained problems with indoor air quality, possibly attributable to fungi, could require much more extensive sampling protocols than described below, including analysis of dust samples for fungi and fungal products, and determination of fungal volatiles in the air.

2. Inspection and Testing Rationale

This protocol is based on two types of analysis: (1) detailed inspection of the building and (2) air sampling. Air sampling for fungi is a complex problem and can be done by a variety of means (2). This protocol involves the use of the RCS Biotest Sampler and similar centrifugal microbial samplers. This type of sampler is easy to use and inexpensive. The RCS sampler has been widely used in research done in both The Netherlands and Canada (3,4). Laboratory studies have demonstrated that this device collects fungal spores in air in a linear fashion related to the absolute value determined by the spore mass and a particle counter. Species with widely different sizes and shapes of spores including *Alternaria alternata, Cladosporium cladosporioides, Paecilomyces varioti* and *Penicillium viridicatum* were collected with approximately equal efficiency (5). The principles outlined can be employed with any sampler, suitable for determining the concentration of spores in air, when used according to the manufacturer's directions.

In outdoor air, phylloplane fungi such as *Cladosporium cladosporioides, C. herbarum, Alternaria alternata, Aureobasidium pullulans, etc.*, predominate in the spring, summer, and fall. Normal indoor airspora reflects the outdoor situation, qualitatively in houses. In large buildings with HVAC systems, the indoor airspora reflects the outdoor airspace, qualitatively, but usually at much lower spore concentrations. This inspection and test protocol is designed to detect significant deviations

from this normal situation with readily available expertise. The RCS analysis portion of the protocol is designed to get a reading of the air spora at the time of sampling. The inspection protocol is designed to find potential sources of fungal contamination.

3. Inspection Protocol

The first step in determining the level of fungal contamination in indoor air is to conduct a visual inspection of the building using the following check lists. There are different lists for small and large buildings. Each item on the list is checked and, if found to be present, appropriate action is taken by one of three recommended courses. The three courses of action are:

A. RCS strips **MUST** be looked at by a mycologist to check for pathogens,

B. RCS strips should, when possible, be looked at by a mycologist, and

C. Determine if there is a source of fungal contamination.

3.a) Checklist for Houses and Small Buildings

Observation	Action
1. birds or bats near air intake of HRV	A
2. birds or bats in attics or basement	A
3. faulty floor drain traps	A
4. toilet vented into building	A
5. contaminated/obviously dirty humidifiers	B

6. dehumidifiers over floor drain in basement	B
7. earthen floor in basement	B
8. attached crawl spaces	B
9. fungal growth on ceiling tiles, walls, windows, etc.	B
10. strong evidence of moldy smells	B
11. many plants, attached greenhouse	C
12. condensation on windows	C
13. raised basement floors	C
14. carpet on concrete	C
15. window air conditioners (contaminated filter)	C
16. dirty HRV filters	C
17. closets adjacent to outside walls	C
18. dusts and filters in house heating/air conditioning dirty	C
19. fungal growth in attics	C
20. landscaping that directs water into house	C
21. trees and shrubs against house	C
22. sink overflows (certain designs only)	C

3.b) Checklist for Large Buildings

Observation	Action
1. evidence of birds or bats near air intake	A
2. evidence of birds or bats in building	A

interior	
3. contaminated humidifiers (including portable units)	A
4. faulty floor drain traps	A
5. sewage drains open	A
6. air intake through crawl space	B
7. evidence of fungal growth on ceiling tiles, walls, etc.	B
8. self-contained heating/cooking units	C
9. catastrophic floods	C
10. indicators for small buildings listed above as appropriate	

4. RCS Biotest Sampler Method

4.a) Materials Required

i) RCS Biotest Sampler. Institut GmbH D 6000, Frankfurt, W. Germany.

ii) RCS Strips, Gelman Science Inc, Montreal, P.Q.

From the manufacturer's recommendations and our experience (including procedures after receipt of samples in the laboratory), the use of 2% malt extract agar Rose bengal strips available from the manufacturer is satisfactory under most circumstances. In cases where specific fungi must be looked for, strips containing selective media can be prepared by a mycology laboratory using blank strips available from the manufacturer.

4. b) RCS Sampling

RCS samples should be taken while air circulation, air conditioner, furnace, ventillation, humidifier, etc., are operating. Basement windows should be closed and, if possible, an upstairs window on the leeward side of the building should be opened. In large buildings, the ventilation system should be operating normally. Special consideration should be given to sampling early on Monday morning if the HVAC system is turned off for the weekend. Each room in a house should be sampled while standing still, holding the sampler at eye level or it should be mounted 1-1.5 m off the ground on a stand. In large buildings, samples should be taken at several locations throughout the desired area including e.g., near air outlets, at desk level, and in the adjacent hallway. Duplicate or triplicate samples can be taken. After sampling, the strips should be kept at 2-5 degrees Celcius and returned to the laboratory within a few hours.

4.c) Operation of the RCS Sampler

1. Remove the plastic cover of the RCS sampler and sterilize the open drum of the instrument with an ethanol swab or a suitable disinfecting solution. This procedure must be done between each house and between each floor in a large building.

2. Tear open, pulling back a few centimeters, the rounded end of the plastic wrapper in which the agar strip is packed. Carefully remove the agar strip without touching the surface of the agar with your fingers. Insert the strip immediately in the slot at the side of the sterilized drum with the agar facing inwards.

Ensure that the strip protrudes enough for it to be removed at the end of the sampling time.

3. Set the sampling time switch to four minutes (6).

4. Place the instrument at the required location and turn on by moving the main switch from 0 to 1 (the light will then go on). Start the instrument by depressing the start button at the bottom of the panel. Do not move the instrument while a sample is being taken; it will stop automatically at the end of the sampling time.

5. At the end of the sampling time, pull out the agar strip without touching the agar with your fingers and place it back in its original wrapper with the agar facing the bulged surface of the wrapper.

6. Seal the open end with ashesive tape, ensuring that it is air-tight. Label it so as not to obstruct the view of the agar.

7. Incubate at 25 degrees Celcius for two to ten days.

8. Counts should be taken daily, commencing with day two.

9. The final count is translated into Colony Forming Units (CFU) per cubic meter using the following equation, based on the time of exposure of the RCS strip and the nornal air flow through the sampler (40 L/min):

$$CFU/m^3 = \frac{\frac{1000\ L/m^3 \times \text{Colony Counts}}{40\ L/min}}{\text{Sampling Time (min)}}$$

Note: other samplers can be used to derive the required information if operated according to manufacturer's instructions.

4.d) RCS Strips Interpretation

If the user has no mycological expertise, all samples showing more than zero Colony Forming Units (CFU) must be referred to a mycologist. If the user has been trained to identify the common phylloplane fungi *Cladosporium* and *Alternaria* and to recognize a few other fungi, approximately 90% of the real situations found in Canadian buildings can be dealt with without reference to a mycologist. There are several circumstances when a professional mycologist must be found to resolve the question of any hazardous situation with respect to fungi in indoor air. These action guidelines are meant to prompt for this investigation only, and do not directly relate to health but, rather reflect deviations from the Canadian norms (4) and require the exercise of good judgement. In general terms, more weight must be placed on the inspection side of the process than the RCS (or other) sampling.

The determination of the RCS value in CFU m^{-3} allows the selection of one of the following appropriate courses of action:

A. RCS value = 0 CFU m^{-3}: no further action unless indicated by inspection,

B. RCS value >= 50 CFU m^{-3} one species only: species and source must be identified to determine further action,

C. RCS value <= 150-200 CFU m^{-3} several to many species present: no further action unless indicated by inspection,

D. RCS value >= 200 CFU m^{-3} several to many species present: prudence requires further investigation,

E. RCS value <= 400-500 CFU m^{-3} mainly *Cladosporium, Alternaria*: no further action unless indicated by inspection, and

F. RCS value > 500 CFU m^{-3} mainly *Cladosporium, Alternaria*: determine reason.

Note: Continuing unexplained complaints may require more detailed analysis for fungi and fungal products in air or dust.

5. Acknowledgements

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